SELF-DEPOLARIZATION AND SELF-QUENCHING IN FLUORESCENT SOLUTIONS

By L. SZALAY, B. SÁRKÁNY and E. TOMBÁCZ

Institute of Experimental Physics, József Attila University, Szeged

(Received January 4, 1965)

The validity of JABŁOŃSKI's theory of self-depolarization has been studied for solutions of fluorescein in water-glycerol mixtures of different viscosity. A phenomenon of rotational repolarization has been found and explained in solutions of extreme high concentration.

1. Self-depolarization has already been treated theoretically by many authors [1]. A simple sphere-model was given by JABŁOŃSKI [2]: the primarily excited luminescent particle A^* is surrounded by an "active sphere". If an unexcited particle of the same kind A exists within the sphere, the probability of radiative deactivation of A^* is equal to the probability of radiationless transfer of exciting energy to A, independently on the distance between A^* and A. In terms of emission anisotropy [5] the calculations led to the result

$$\frac{r_0}{r} = \frac{\sum_{k=1}^{\infty} \frac{v^{k-1}}{k!} \frac{k}{\gamma + W_k + W_f}}{\sum_{k=1}^{\infty} \frac{v^{k-1}}{k!} \left(\frac{1}{\gamma + W_k + W_f} + \frac{k-1}{\gamma + W_k + W_f + k\mu} \right)}, \tag{1}$$

where the notations are as follows: r — emission anisotropy of fluorescence; γ, W_k, W_f and μ — the probability of emission, self-quenching, inner quenching + foreign quenching and radiationless transition, respectively; v — the number of luminescent particles in the active sphere of a volume v ($v = 6{,}02 \cdot 10^{20} v c_M$; c_M is the concentration of the solution in mole/l); r_0 — the limiting value of emission anisotropy ($r \to r_0$, if $c_M \to 0$).

For $W_k = W_f = 0$ (*i. e.*, no self-quenching and foreign quenching exist) and $\gamma = \mu$, the relation

$$\frac{r_0}{r} = \frac{v^2}{2(v - 1 + e^{-v})} \tag{2}$$

is obtained and found to be valid in the range of small concentrations, where the assumption $W_k = 0$ is reliable. Under special assumptions a formula for higher

concentrations has been derived from eq. (1) by BOJARSKI [3].

$$\frac{r_0}{r} = \frac{v^2 \frac{s}{s+1} g_s(v)}{\left[v - 1 + e^{-v} - \frac{v^2}{s+1} g_s(v)\right]} \tag{3}$$

where $s \equiv \mu/W_1 \gg 1$, *i. e.*, the probability of inner quenching (W_1) is much smaller, than that of radiationless energy transfer (μ), and $g_s(v) \equiv {}_1F_1(1, s+2, -v)$, a given value of the confluent hypergeometric series [20]. Though for small v and large s $g_s(v)$ is rapidly convergent, in cases of the most important highest concentrations the convergency is very slow.

A refinement of the sphere-model (introducing shells of the same volume and a dependence of the radiationless transfer of energy on the distance of the particles) given by BOJARSKI [4] yields a more complex expression even for the case of $W_k = 0$:

$$\frac{r_0}{r} = \frac{1 + \sum_{k=1}^{z} \frac{k_l}{l^2}}{2e^{-vz} \sum_{l_1 \ldots l_z} \frac{v^{k_1 - 1}}{(k_1 - 1)!} \prod_{l=z}^{z} \frac{v^{k_l}}{k_l!}}, \tag{4}$$

where z denotes the number of shells considered. Though the cases when $z \geqq 4$ may be practically neglected, the calculations with eq. (4) are tedious and the results are not in good agreement with the experiments for higher concentrations.

2. An approximation for high concentrations may be given as follows. Let eq. (1) be rewritten with the notations $W \equiv \gamma + W_k + W_f$ and $M \equiv \mu/W$:

$$\frac{r}{r_0} = \frac{\sum_{k=1}^{\infty} \frac{v^{k-1}}{k!} k \frac{M+1}{kM+1}}{\sum_{k=1}^{\infty} \frac{v^{k-1}}{k!} k}. \tag{5}$$

Since $\sum_{k=1}^{\infty} \frac{v^{k-1}}{k!} k = e^{-v}$,

$$\frac{r}{r_0} = (M+1)e^{-v} \sum_{k=1}^{\infty} \frac{v^{k-1}}{(k-1)!} \frac{1}{kM+1}. \tag{6}$$

Supposing there is no foreign quenching ($W_f = 0$) $M = \mu/(\gamma + W_k)$. For $\mu = \gamma$ (as before in eq. (2)) and using the relations $\mu = 1/\tau_0$ and $\gamma + W_k = 1/\tau$ (where τ_0 and τ denote the fluorescence in unquenched and quenched solutions) $M = \tau/\tau_0$. Substituting this value of M into eq. (6), the relation

$$\frac{r}{r_0} = \left(\frac{\tau}{\tau_0} + 1\right) e^{-v} \sum_{k=1}^{\infty} \frac{v^{k-1}}{(k-1)!} \frac{1}{k \frac{\tau}{\tau_0} + 1} \tag{7}$$

holds.

A simple lower limit may be given for r/r_0. Apparently

$$\frac{r}{r_0} = e^{-\nu} \sum_{k=1}^{\infty} \frac{\nu^{k-1}}{(k-1)!} \frac{1}{1+(k-1)\frac{M}{M+1}} > e^{-\nu} \sum_{k=1}^{\infty} \frac{\nu^{k-1}}{(k-1)!} \frac{1}{\left(1+\frac{M}{M+1}\right)^{k-1}} =$$

$$= e^{-\nu} \sum_{k=1}^{\infty} \frac{\left[\frac{\nu}{1+\frac{M}{(M+1)}}\right]^{k-1}}{(k-1)!} = e^{-\nu} \cdot e^{\frac{\nu}{1+M/(M+1)}} = e^{-M\nu/(1+2M)},$$

i. e.

$$\frac{r}{r_0} > e^{-\frac{\tau/\tau_0}{1+2\tau/\tau_0}\nu}. \tag{8}$$

Instead of inequality (8) — as it will be seen — eq. (7) may be more satisfactorily approximated by the equation

$$\frac{r}{r_0} = \frac{\tau/\tau_0+1}{(\tau/\tau_0)^2} \frac{\nu\tau/\tau_0-1+e^{-\nu}}{\nu^2} \tag{9}$$

or, if $\tau/\tau_0 = \eta/\eta_0$ (the relative quantum yield)

$$\frac{r}{r_0} = \frac{\eta/\eta_0+1}{(\eta/\eta_0)^2} \frac{\nu\eta/\eta_0-1+e^{-\nu}}{\nu^2}. \tag{10}$$

For unquenched solutions ($\tau/\tau_0 = \eta/\eta_0 = 1$) eqs. (9) and (10) become identical with eq. (2).

3. Fluorescein solutions containing glycerol and water in different proportions and 2 per cent NaOH were studied. The concentration of dye was varied between $1{,}0\cdot10^{-5} - 5{,}0\cdot10^{-2}$ mole/l. The photoelectric apparatus used for measuring the degree of polarization is described in [6]. The wavelength of the exciting light was 510 mμ, the temperature of the samples was maintained at a value of $30{,}0 \pm 0{,}1$ centigrade by means of a Höppler ultrathermostat. The viscosity was measured at the same temperature by a Höppler viscosimeter. The degree of polarization of fluorescence obtained experimentally was corrected for secondary fluorescence according to an equation and method given in [7]. For this correction the absorption and emission spectra were measured by a photoelectric spectrophotometer Optica Milano CF-4. The absolute quantum yield of fluorescence was determined by a method given in [8].

Fig. 1 exhibits the depolarization curves for solutions of different viscosities. As it is to be seen a considerable repolarization occurs in the concentration range of $1{,}0\cdot10^{-2} - 5\cdot10^{-2}$ mole/l. The repolarization increases with decreasing viscosity. An opposite behaviour would be expected because of the enhanced rotational possibility of particles in solutions of small viscosity. If, however, the self-quenching curves shown in Fig. 2 for glycerol and (after [8]) for water solvent are considered, it may be concluded that in water a shorter mean life time of the excited state should prevail than in glycerol, consequently — in spite of the high rotational

mobility of fluorescent particles — is solutions of small viscosity a higher repolarization may appear than in solutions of higher viscosity.

As for the quantitative agreement of eq. (10) and the experiment Fig. 3 shows the self-depolarization curve for the whole range of concentration and in the case of the solution of highest viscosity. The empirical parameter v, the volume of the active sphere, was determined by fitting the experimental values of r to eq. (10) in the range of small concentrations. The radius of the active sphere was found to

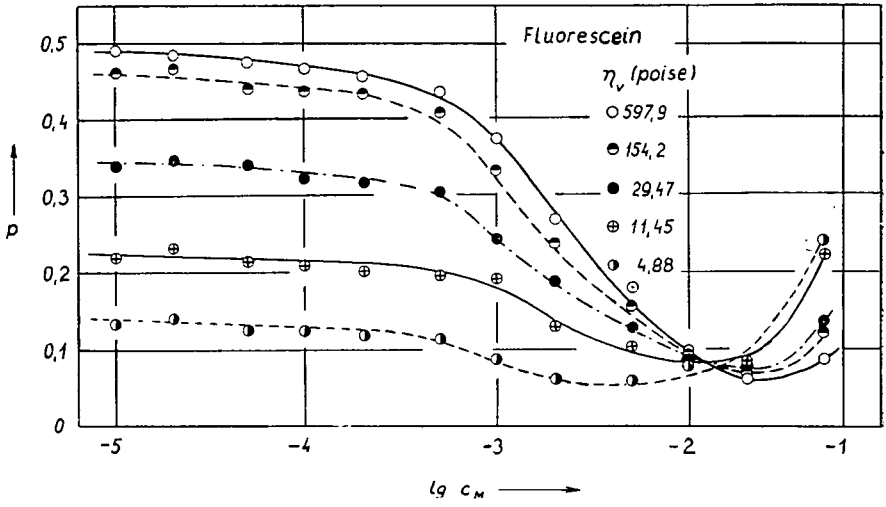

Fig. 1

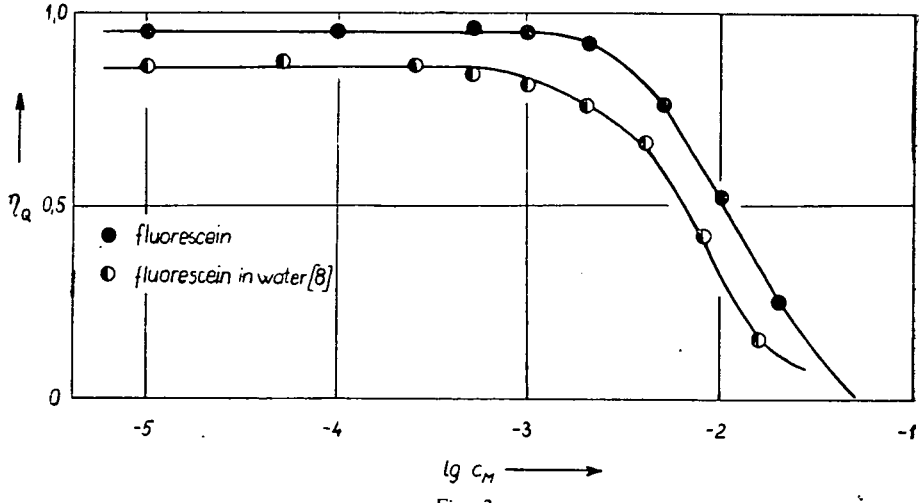

Fig. 2

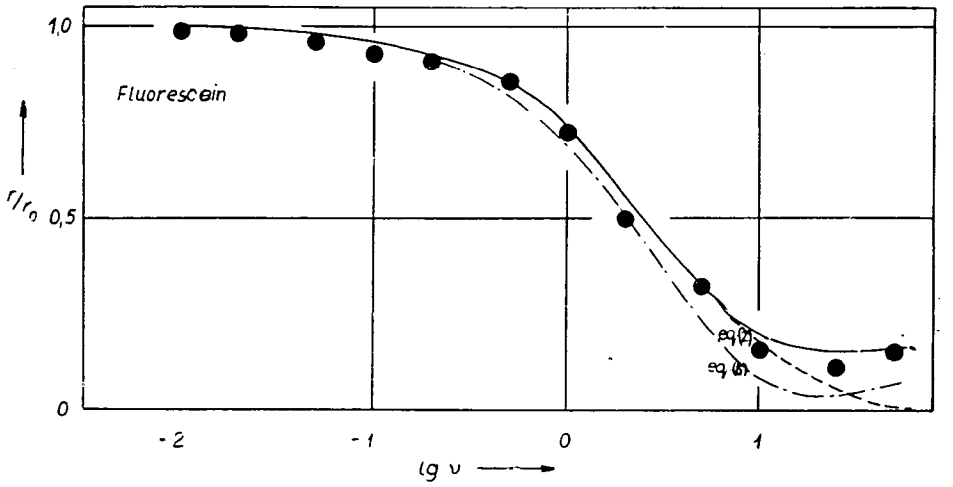
1,0
Fluorescein
r/r0
0,5
0
-2
-1
0
1
lg ν
eq (2)
eq (6)

Fig. 3

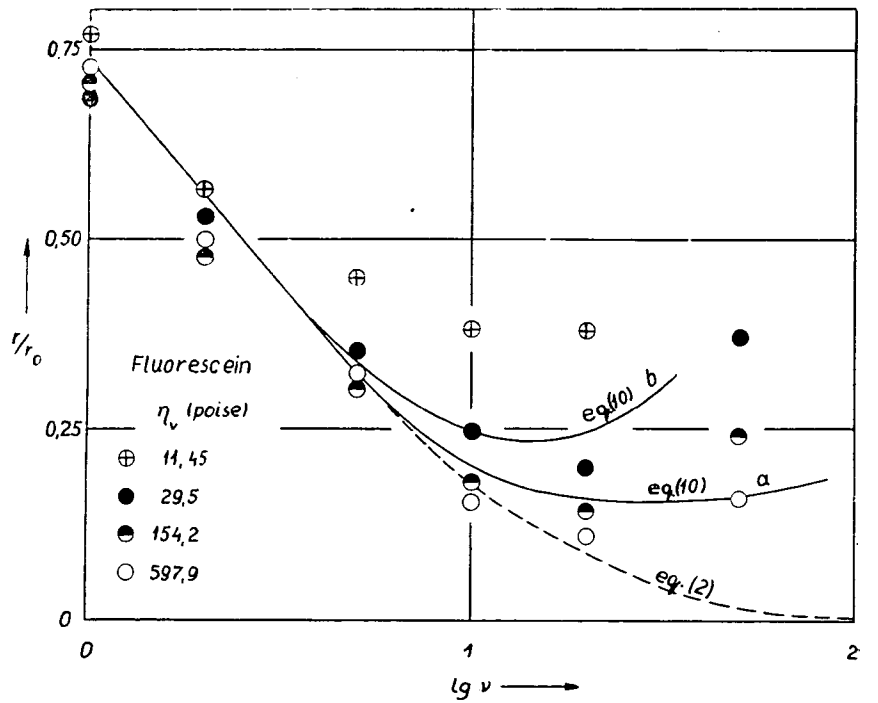
0,75
0,50
r/r0
0,25
0
Fluorescein
ηv (poise)
11,45
29,5
154,2
597,9
eq (10) b
eq (10) a
eq (2)
0
1
2
lg ν

Fig. 4

be $R_J = 72{,}0$ Å (a value near to 70,8 Å given in [9] and 79 Å given in [10] for the same system). The broken line, dotted line and the solid line were calculated by eq. (2), (8) and (10), respectively. The best agreement of experiment and theory is given by eq. (10). Fig. 4. represents the theoretical curves of self-depolarization calculated by eq. (2) (broken line) and by eq. (10) (solid lines) for the concentration range of $1{,}0 \cdot 10^{-3} - 5 \cdot 10^{-2}$ mole/l. The relative yield values were taken from the data in Fig. 2. The upper and lower depolarization curves (solid lines) in Fig. 4. belong to water and glycerol solutions, respectively. The experimental points lie near to these curves which can account — at least partly — for the repolarization as well.

In case of trypaflavine dissolved in a mixture of glycerol-ethanol and containing 2 per cent acetic acid the data for self-depolarization and self-quenching have already been published [11]. Fig. 5 shows how eqs. (2), (8) and (10) are fulfilled for this system. The radius of active sphere was found to be $R_J = 42{,}8$ Å (in [10] $R_J = 40$ Å), with this parameter and with $\eta/\eta_0 = 0{,}90$, 0,25 and 0,16 for the three highest concentrations we obtain the broken, dotted and solid line by eqs. (2), (8) and (10), respectively. According to [11] these quantum yields are too high, probably, eq. (7) should give a better agreement, because usually $\tau/\tau_0 < \eta/\eta_0$ for higher concentrations. Because of lack of experimental τ/τ_0-values it was not possible to check eq. (7).

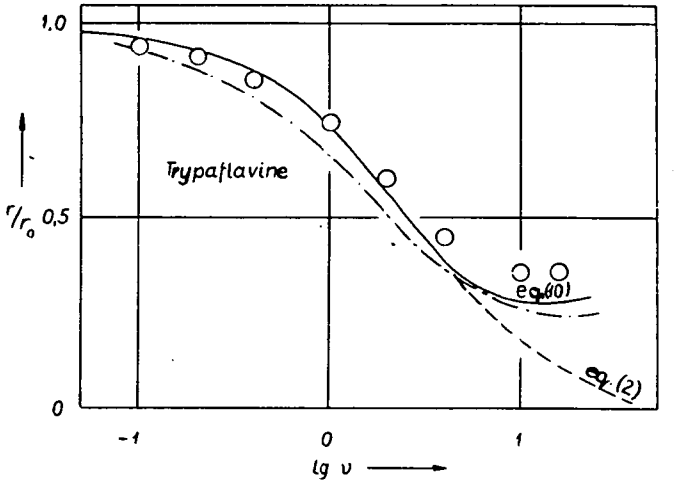

Fig. 5

The recent measurements referring to the fluorescein solutions of different η_v viscosities corroborate the statements given in [14] about the independence of the processes determining the rotational depolarization and the self-depolarization. As Fig. 6 shows the self-depolarization curves belonging to different concentrations get nearer to each other, if the viscosity is decreased, since in the competition of the two processes of depolarization the role of rotational depolarization becomes prevailing. However, in solutions of very high concentration this regularity is no

longer to be found. The increase of degree of polarization of fluorescence with the decrease of the viscosity of solution (shown by broken line for a concentration of $5 \cdot 10^{-2}$ mole/l in Fig. 6) — some kind of rotational repolarization — is not in contradiction with the well-known PERRIN—LEVSHIN equation (see *e. g.* in [15]). Namely, according to this equation the increase of the degree of polarization of fluorescence — *ceteris paribus* — is expected, when τ/η_v is decreased and this quantity may decrease — due to the dependence of self-quenching on the viscosity shown in Fig. 2 — even if η_v decreases.

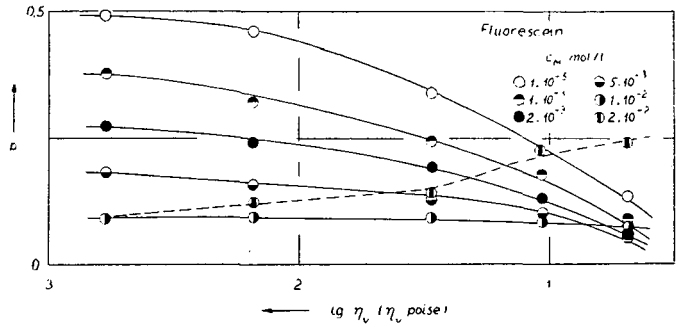

Fig. 6

The independence of the two mentioned depolarization processes led to the reasoning that PERRIN—LEVSHIN equation must no be applied to self-depolarization [14]. In [16], however, a formula was derived from this equation for the self-depolarization. This formula implies a mean life time of the excited state obtained by a relation for self-quenching and yield an increase of the degree of polarization of fluorescence with the increase of concentration in the range of low concentrations, a result inconsistent with the experimental results obtained hitherto. (The alone case mentioned in [17], except that in [16], could not be corroborated in [18].) Consequently, our results referring to the independence of rotational depolarization and self-depolarization show that the formula given in [16] may not be considered as reliable in principle. The experimentally obtained rise of the degree of polarization of fluorescence at small concentrations should be carefully controlled for the systems mentioned in [16].

* * *

Sincere thanks are due to Prof. Dr. A. BUDÓ, the Head of the Institute for the valuable discussions and the help in this work.

References

[1] *Vavilov, S. J.:* Zh. eksp. teor. fiz. **13**, 13 (1943).
Förster, Th.: Ann. Phys. **2**, 55 (1948); Z. Naturfschg. **4a**, 321 (1949).
Galanin, M. D.: Zh. eksp. teor. fiz. **28**, 485 (1955).
Weber, G.: Trans. Farad. Soc. **50**, 552 (1954).

Ketskeméty, I.: Acta Phys. et Chem. Szeged, **1**, 29 (1955).
Ore, A.: J. Chem. Phys. **31**, 442 (1959).
[2] *Jabłoński, A.:* Acta Phys. Polon. **14**, 295 (1955); **17**, 481 (1958).
[3] *Bojarski, C.:* Ann. Phys. **8**, 402 (1961).
[4] *Bojarski, C.:* Bull. Acad. Polon. **6**, 719 (1958).
[5] *Jabłoński, A.:* Acta Phys. Polon. **16**, 471 (1957); Bull. Acad. Polon. **10**, 555 (1962).
[6] *Ketskeméty, I., L. Gargya, E. Salkovits:* Acta Phys. et Chem. Szeged **3**, 16 (1957).
[7] *Budó, Á., I. Ketskeméty:* Acta Phys. Hung. **14**, 167 (1962).
[8] *Budó, Á., J. Dombi, L. Szöllősy:* Acta Phys. et Chem. Szeged **2**, 18 (1956).
[9] *Szalay, L., B. Sárkány:* Acta Phys. et Chem. Szeged **8**, 25 (1962).
[10] *Kawski, A.:* Z. Naturfschg. **18a**, 961 (1963).
[11] *Szalay, L.:* Ann. Phys. **14**, 221 (1964).
[12] *Rohatgi, K. K.:* Z. Phys. Chem. **217**, 353 (1961).
[13] *Budó, Á., L. Szalay:* Z. Naturfschg. **18a**, 90 (1963).
[14] *Pheofilov, P. P., B. J. Shvesnikov:* J. Phys. USSR **3**, 493 (1960)
[15] *Pringsheim, P.:* Fluorescence and Phosphorescence (Interscience Publ. New York, London 1949).
[16] *Chaudhuri, K. D.:* Z. Phys. **154**, 34 (1959).
[17] *Czajkowski, M., J. Grzywac:* Bull. Acad. Polon. **6**, 107 (1958).
[18] *Glowacki, J., A. Kawski, B. Polacka:* Bull. Acad. Polon. **7**, 353 (1959).
[19] *Ryshik, Gradstein:* Tafeln $\Sigma\Pi\int$ Tables (VEB Deutscher Verlag der Wissenschaften, Berlin, 1957. p. 397).

КОНЦЕНТРАЦИОННАЯ ДЕПОЛЯРИЗАЦИЯ И КОНЦЕНТРАЦИОННОЕ ТУШЕНИЕ ФЛУОРЕСЦЕНЦИРУЮЩИХ РАСТВОРОВ

Л. Салаи, Б. Шаркань, Э. Томбац

Исследовалось выполнение концентрационной деполяризованной теории Яблонского на разных вязких глицеринно- водных растворах флуоресцениа. В случае больших концентрационных растворов обнаруживается явление вращательной реполяризации.